REMARKS

I. Status of the Claims

This Request for Continued Examination and Amendment is being submitted in response to the final Office Action dated July 2, 2009 in the above-identified application. Concurrently with this Amendment, Applicant submits a petition for a three-month extension of time for filing a response, along with the requisite fee. Therefore the time for filing a response to the July 2, 2009 Office Action is thereby extended to January 2, 2010, and this Amendment is being timely filed. If it is determined that any additional fee is due in connection with this filing, the Commissioner is authorized to charge said fees to Deposit Account No. 50-0552.

Claims 29 and 34 have been amended without prejudice. Support for these amendments can be found, for example, on page 5, lines 20 to 22, and page 51, lines 13 to 19 of the specification as filed.

Claims 1 to 28 were previously canceled without prejudice via preliminary amendment dated October 10, 2006. Claims 33 and 35 have been canceled without prejudice in the present Amendment.

Claims 29 to 32, 34, 36 and 37 are now pending.

Applicants respectfully submit that no new matter has been added by virtue of this amendment.

II. Claim Rejections- 35 USC §102

In the Office Action, the Examiner maintained the rejection of claims 29 to 32, 34, 36 and 37 under 35 U.S.C. 102(b) as being anticipated by Yue et al. (WO/2002/026982).

Independent claim 29, as amended, recites: "A method of diagnosing cancer comprising detecting human C20orf102 protein present in blood, serum or plasma."

Independent claim 34, as amended, recites: "A method of diagnosing cancer comprising the steps of: (a) collecting blood, serum or plasma from a subject; and (b) detecting human C20orf102 protein contained in the collected sample."

As shown in the amended independent claims 29 and 34, the present invention is directed to a method of diagnosing cancer comprising detecting human C20orf102 protein present in blood, serum or plasma. The Yue et al. reference does not disclose a method for detecting C20orf102 protein in blood, serum or plasma. As admitted in the Office Action on page 5, line 12, "Yue et al. does not teach a sample from a subject comprising blood serum or plasma."

Therefore, Applicants submit that the Yue et al. reference does not show or teach "A method of diagnosing cancer comprising detecting human C20orf102 protein present in blood, serum or plasma" as recited in claim 29. The Yue reference also does not show or teach "A method of diagnosing cancer comprising the steps of: (a) collecting blood, serum or plasma from a subject; and (b) detecting human C20orf102 protein contained in the collected sample" as recited in claim 34.

Accordingly, independent claims 29 and 34 are not anticipated by the Yue et al. reference.

For the foregoing reasons, withdrawal of the rejection under 35 U.S.C. § 102(b) to claims 29 and 34, as well as, dependent claims to 30 to 32, 36 and 37is respectfully requested.

III. <u>Claim Rejections- 35 USC §103</u>

In the Office Action, the Examiner maintained the rejection of claims 29 to 37 under 35 U.S.C. 103(a) as being obvious over Yue et al. (WO/2002/026982) in view of Ruben et al. (U.S. Patent 7,169,565).

Secreted proteins are not limited extracellular proteins

In the Office Action, the Examiner alleges *inter alia* that detecting C20orf102 protein in a sample such as blood, serum or plasma is prima facie obvious from Yue et al. in view of Ruben et al. which discloses general techniques for detecting polypeptides in biological analytes. See Office Action, page 5, lines 17-20. Applicant respectfully submits that Yue et al. teaches that the molecule of SEO ID NO:3 (polypeptide) has 51% local homology with a mouse transmembrane protein (GenBank ID g7259265). This means that the C20orf102 protein has 51% local homology with a transmembrane protein and is suggested to be anchored on a cell surface.

Ruben et al. discloses a method for detecting a protein present in free form in blood, serum or plasma. In view of the different nature of the two proteins, a person skilled in the art would not have been motivated to detect a transmembrane protein anchored on a cell suggest using the method for detecting a protein present in free form in a sample. Thus, a person skilled in the art would have no reason to combine the teaching of Yue et al. with the teaching of Ruben et al.

Therefore, Applicants submit that neither the Yue et al. reference nor the Ruben reference show or teach "A method of diagnosing cancer comprising detecting human C20orf102 protein present in blood, serum or plasma" as recited in claim 29. Applicants also submit that neither the Yue et al. reference nor the Ruben reference show or teach "A method of diagnosing cancer comprising the steps of: (a) collecting blood, serum or plasma from a subject; and (b) detecting human C20orf102 protein contained in the collected sample" as recited in claim 34.

In the Office Action, the Examiner states in response to the argument filed on April 9, 2009 that "The molecules disclosed in Yue et al. are human secreted proteins (SCEP) and therefore would be found in bodily fluids such as blood, plasma or serum. --- Ruben et al. disclose bodily fluids such as blood, plasma or serum can be used In a method to detect polypeptide levels using an antibody directed to said polypeptide." See Office Action, page 6, lines 11-15.

Applicant respectfully submits that the Examiner's reasoning appears to be based on a deliberate interpretation of the term "secreted protein" as an extracellular protein secreted out of the cells. Applicant respectfully submits that such an interpretation would be unjustified because the term "secreted protein" originally has a broad meaning, including both proteins secreted out of the cells and those anchored on the membrane.

Applicant respectfully submits that, it is clear from the description of Yue et al. cited below (See Yue et al. at page 83, line 28 to page 84, line 5; and page 84, line 27 to page 85, line 4) that the "secreted protein" has a broader meaning including proteins anchored on a cell surface, such as membrane proteins:

> "Alternatively, an assay for SECP activity measures the amount of SECP in secretory, membrane-bound organelles. Transfected cells as described above are harvested and lysed. The lysate is fractionated using methods known to those of skill in the art, for example, sucrose gradient ultracentrifugation. Such methods allow the isolation of subcellular components such as the Golgi apparatus, ER, small membrane-bound vesicles, and other secretory organelles.
> Immunoprecipitations from fractionated and total cell lysates are performed using SECP-specific antibodies, and immunoprecipitated samples are analyzed using SDS-PAGE and immunoblotting techniques. The concentration of SECP in secretory organelles relative to SECP in total cell lysate is proportional to the amount of SECP in transit through the secretory pathway.
> Alternatively, AMP binding activity is measured by combining SECP with 32P-labeled AMP. The reaction is incubated at 37°C and terminated by addition of trichloroacetic acid. The acid extract is neutralized and subjected to gel electrophoresis to remove unbound label. The radioactivity retained in the gel Is proportional to SECP activity."
> **See Yue et al. at page 83, line 28 to page 84, line 5.**
>
> "Alternatively, an assay for SECP activity measures the expression of SECP on the cell surface. cDNA encoding SECP is transfected into a non-leukocytic cell line. Cell surface proteins are labeled with biotin (de la Fuente, M. A. et al. (1997) Blood 90: 2398-2405). Immunoprecipitations are performed using SECP-specific antibodies, and immunoprecipitated samples are analyzed using SDS-PAGE and immunoblotting techniques. The ratio of labeled immunoprecipitant to unlabeled immunoprecipltant is proportional to the amount of SECP expressed on the cell surface.
> Alternatively, an assay for SECP activity measures the amount of cell aggregation induced by overexpression of SECP. In this assay, cultured cells such as NIH3T3 are transfected with cDNA encoding SECP contained within a suitable mammalian expression vector under control of a strong promoter. Cotransfection with cDNA encoding a fluorescent marker protein, such as Green Fluorescent Protein (CLONTECH),

> is useful for Identifying stable transfectants. The amount of cell agglutination, or clumping, associated with transfected cells is compared with that associated with untransfected cells. The amount of cell agglutination is a direct measure of SECP activity."
> **See Yue et al. at page 84, line 27 to page 85, line 4.**

In addition, Yue et al. describes that 22 molecules out of 67 molecules shown in Table 3 as "secreted protein" have a transmembrane domain in its motif. Applicant respectfully submits that such a description clearly indicates that the "secreted protein" disclosed in Yue et al. is used in its broader meaning where a cell surface anchored protein such as a membrane protein are also be encompasses by the term •secreted protein."

Accordingly, Yue et al. suggests that C20orf102 is a protein anchored on a cell surface but is not a protein secreted out of the cell. In view of the different nature of the two proteins, a person skilled in the art could not combine the C20orf102 protein anchored on a cell surface as disclosed in Yue et al. and a method for detecting a protein present in free form in blood, serum or plasma as disclosed in Ruben et al.

Applicant respectfully direct the Examiner's attention to the publication "Essential Cell Biology, 2nd Ed. p. 516-523", referred herein as "Reference" and a copy of which is attached herewith as Appendix A, which further supports applicant's discussion above. As clearly demonstrated in page 522, lines 1-10 of the Reference, those skilled in the art understood that the use of the term "secretion" is not limited to those molecules secreted out of the cells.

Moreover, Applicant respectfully suggests that Yue et al. only discloses expression of the gene but not the expression of the polypeptides. Yue et al. discloses expression of the gene coding for the C20orf102 protein represented by SEQ ID NO: 3 (polypeptide) which is said to have 51 % local homology with a mouse transmembrane protein (GenBank ID g7259265), but fails to show expression of the C20orf102 protein. It was well established in the art that the expression level of a gene and the expression level of the corresponding protein are not always consistent.

Accordingly, a person skilled in the art could not have reasonably expected that the C20orf102 protein is expressed in cancer cells based on the disclosure of Yue et al. which merely discloses expression of the gene but fails to show the expression of the protein.

Therefore, Applicants submit that neither the Yue et al. reference nor the Ruben reference show or teach "A method of diagnosing cancer comprising detecting human C20orf102 protein present in blood, serum or plasma" as recited in claim 29. Applicants also submit that neither the Yue et al. reference nor the Ruben reference show or teach "A method of diagnosing cancer comprising the steps of: (a) collecting blood, serum or plasma from a subject; and (b) detecting human C20orf102 protein contained in the collected sample" as recited in claim 34.

In view of the above, the invention claimed in the amended claim is not obvious from Yue et al. in view of Ruben et al.

For the foregoing reasons, withdrawal of the rejection to claims 29 to 37 under 35 U.S.C. § 103(a) is respectfully requested.

Conclusion

An early and favorable action on the merits is earnestly solicited. According to currently recommended Patent Office policy, the Examiner is requested to contact the undersigned in the event that a telephonic interview will advance the prosecution of this application.

Respectfully submitted,
DAVIDSON, DAVIDSON & KAPPEL, LLC
By: Sunil Raval
Sunil Raval
Reg. No. 47,886

DAVIDSON, DAVIDSON & KAPPEL, LLC
485 Seventh Avenue, 14th Floor
New York, NY 10018
(212) 736-1940

APPENDIX A

essential cell biology

second edition

Bruce Alberts • Dennis Bray
Karen Hopkin • Alexander Johnson
Julian Lewis • Martin Raff
Keith Roberts • Peter Walter

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Essential Cell Biology 2 Interactive
Artistic and Scientific Direction: Peter Walter
Narrated by: Julie Theriot
Production Design and Development: Michael Morales

Bruce Alberts received his Ph.D. from Harvard University and is President of the National Academy of Sciences and Professor of Biochemistry and Biophysics at the University of California, San Francisco. **Dennis Bray** received his Ph.D. from Massachusetts Institute of Technology and is currently a Medical Research Council Fellow in the Department of Zoology, University of Cambridge. **Karen Hopkin** received her Ph.D. in biochemistry from the Albert Einstein College of Medicine in the Bronx and is a science writer in Cambridge, Massachusetts. **Alexander Johnson** received his Ph.D. from Harvard University and is a Professor of Microbiology and Immunology and Co-Director of the Biochemistry and Molecular Biology Program at the University of California, San Francisco. **Julian Lewis** received his D.Phil. from the University of Oxford and is a Principal Scientist at the London Research Institute of Cancer Research UK. **Martin Raff** received his M.D. from McGill University and is at the Medical Research Council Laboratory for Molecular Cell Biology and Cell Biology Unit and in the Biology Department at University College London. **Keith Roberts** received his Ph.D. from the University of Cambridge and is Associate Research Director at the John Innes Centre, Norwich. **Peter Walter** received his Ph.D. from The Rockefeller University in New York and is Professor and Chairman of the Department of Biochemistry and Biophysics at the University of California, San Francisco, and an Investigator of the Howard Hughes Medical Institute.

Published by Garland Science, a member of the Taylor & Francis Group, 29 West 35th Street, New York, NY 10001-2299, USA and 4 Park Square, Milton Park, Abingdon, OX14 4RN, UK.

Printed in Spain.

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging-in-Publication Data
Essential cell biology / Bruce Alberts ... [et al.].-- 2nd ed.
p. cm.
ISBN 0-8153-3480-X.—ISBN 0-8153-3481-8 (pbk.)
1. Cytology. 2. Molecular biology. 3. Biochemistry. I. Alberts, Bruce.
QH581.2.E78 2003
571.6—dc21

2003011505

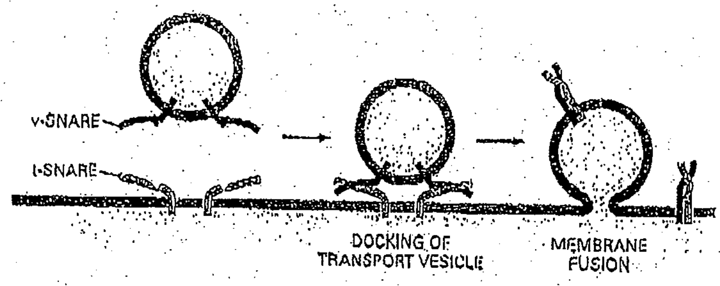


Figure 15–21 SNARE proteins play a central role in membrane fusion. Pairing of v-SNAREs and t-SNAREs forces the two lipid bilayers into close apposition. Lipids then flow between the two bilayers and the membranes fuse. In a cell, other proteins recruited to the fusion site presumably cooperate with SNAREs to initiate fusion. Additional proteins help to pry the SNAREs apart.

fusion complex that provides the means to cross this energy barrier. The SNARE proteins themselves are thought to play a central role in the fusion process: after pairing, v-SNAREs and t-SNAREs wrap around each other, thereby acting like a winch that pulls the two membranes into close proximity (Figure 15–21).

Secretory Pathways

Vesicular traffic is not confined to the interior of the cell. It extends to and from the plasma membrane. Newly made proteins, lipids, and carbohydrates are delivered from the ER, via the Golgi apparatus, to the cell surface by transport vesicles that fuse with the plasma membrane in a process called **exocytosis**. Each molecule that travels along this route passes through a fixed sequence of membrane-enclosed compartments and is often chemically modified en route.

In this section we follow the outward path of proteins as they travel from the ER, where they are made and modified, through the Golgi apparatus, where they are further modified and sorted, to the plasma membrane. As a protein passes from one compartment to another, it is monitored to check that it has folded properly and assembled with its appropriate partners, so that only correctly built proteins are released at the cell surface, while all of the others are degraded in the cell.

Most Proteins Are Covalently Modified in the ER

Most proteins that enter the ER are chemically modified there. Disulfide bonds are formed by the oxidation of pairs of cysteine side chains (see Figure 4–29), a reaction catalyzed by an enzyme that resides in the ER lumen. The disulfide bonds help to stabilize the structure of those proteins that may encounter changes in pH and degradative enzymes outside the cell—either after they are secreted or after they are incorporated into the plasma membrane. Disulfide bonds do not form in the cytosol, because of the reducing environment there.

Many of the proteins that enter the ER lumen or ER membrane are converted to glycoproteins in the ER by the covalent attachment of short oligosaccharide side chains. This process of *glycosylation* is carried out by glycosylating enzymes found in the ER but not in the cytosol. Very few proteins in the cytosol are glycosylated, and those that are have only a single sugar residue attached to them. The oligosaccharides on proteins serve various functions, depending on the protein. They can protect the protein from degradation, hold it in the ER until it is properly folded, or help guide it to the appropriate organelle by serving as a transport signal for packaging the protein into appropriate transport vesicles (as in the case of lysosomal proteins discussed later). When displayed on the cell surface, oligosaccharides form part of the cell's carbohydrate layer (see Figure 11–32) and can function in the recognition of one cell by another.

In the ER, individual sugars are not added one-by-one to the protein to create the oligosaccharide side chain. Instead, a preformed, branched oligosaccharide containing a total of 14 sugars is attached en bloc to all proteins that carry the appropriate site for glycosylation. The oligosaccharide is originally attached to a specialized lipid, called *dolichol*, in the ER membrane; it is then transferred to the amino (NH_2) group of an asparagine side chain on the protein immediately after the target asparagine emerges in the ER lumen during protein translocation (Figure 15–22). The addition takes place in a single enzymatic step catalyzed by a membrane-bound enzyme (an oligosaccharide protein transferase) that has its active site exposed on the luminal side of the ER membrane, which explains why cytosolic proteins are not glycosylated in this way. A simple sequence of three amino acids, of which the asparagine is one, defines which asparagine residues in a protein receive the oligosaccharide. Oligosaccharide side chains linked to an asparagine NH_2 group in a protein are said to be *N-linked* and are by far the most common type of linkage found on glycoproteins.

The addition of the 14-sugar oligosaccharide in the ER is only the first step in a series of further modifications before the mature glycoprotein emerges at the other end of the outward pathway. Despite their initial similarity, the *N*-linked oligosaccharides on mature glycoproteins are remarkably diverse. All of the diversity results from extensive modification of the original precursor structure shown in Figure 15–22. This *oligosaccharide processing* begins in the ER and continues in the Golgi apparatus.

Question 15–6

Why might it be advantageous to add a preassembled block of 14 sugar residues to a protein in the ER, rather than building the sugar chains step-by-step on the surface of the protein by the sequential addition of sugars by individual enzymes?

Exit from the ER Is Controlled to Ensure Protein Quality

Some proteins made in the ER are destined to function there. They are retained in the ER (and are returned to the ER when they escape to the Golgi apparatus) by a C-terminal sequence of four amino acids called an *ER retention signal* (see Table 15–3, p. 504), which is recognized by a membrane-bound receptor protein in the ER and Golgi apparatus. Most proteins that enter the ER, however, are destined for other locations;

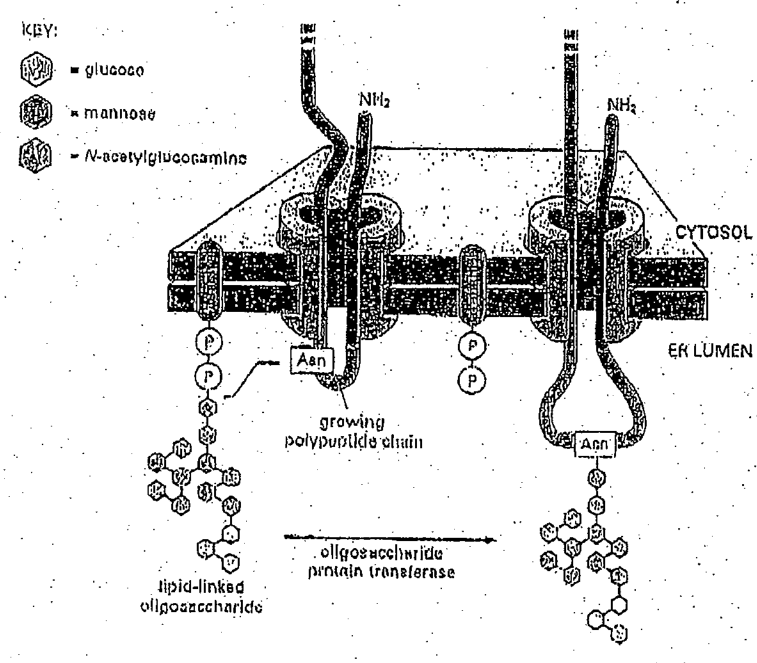


Figure 15–22 Many proteins are glycosylated in the ER. Almost as soon as the polypeptide chain enters the ER lumen, it is glycosylated by addition of oligosaccharide side chains to particular asparagines in the polypeptide. Each oligosaccharide chain is transferred as an intact unit to the asparagine from a lipid called dolichol. Asparagines that are glycosylated are always present in the tripeptide sequences asparagine-X-serine or asparagine-X-threonine, where X can be any amino acid.

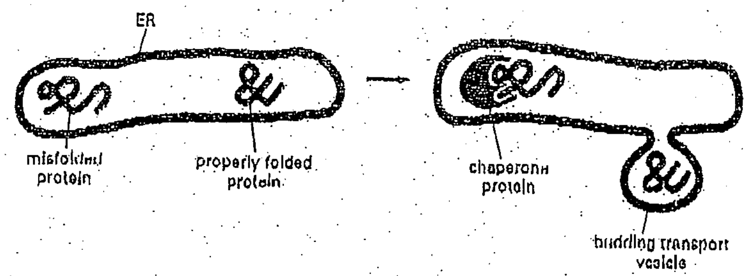


Figure 15–23 Chaperones prevent misfolded or partially assembled proteins from leaving the ER. Misfolded proteins bind to chaperone proteins in the ER lumen and are thereby retained, whereas normally folded proteins are transported in transport vesicles to the Golgi apparatus. If the misfolded proteins fail to refold normally, they are transported into the cytosol, where they are degraded.

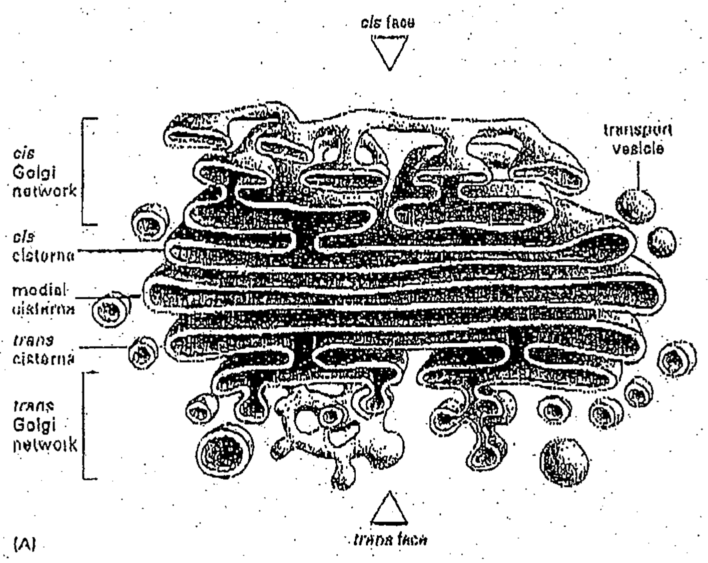
they are packaged into transport vesicles that bud from the ER and fuse with the Golgi apparatus. Exit from the ER, however, is highly selective. Proteins that fold up incorrectly, and dimeric or multimeric proteins that fail to assemble properly, are actively retained in the ER by binding to chaperone proteins that reside there. Interaction with chaperones holds the proteins in the ER until proper folding occurs; otherwise, the proteins are ultimately degraded (Figure 15–23). Antibody molecules, for example, are composed of four polypeptide chains (see Figure 4–32) that assemble into the complete antibody molecule in the ER. Partially assembled antibodies are retained in the ER until all four polypeptide chains have assembled; any antibody molecule that fails to assemble properly is ultimately degraded. In this way the ER controls the quality of the proteins that it exports to the Golgi apparatus.

Sometimes, however, this quality-control mechanism can be detrimental to the organism. The predominant mutation that causes the common genetic disease *cystic fibrosis*, which causes severe degeneration of the lung, for example, produces a plasma-membrane transport protein that is slightly misfolded; even though the mutant protein could function normally as a chloride channel if it reached the plasma membrane, it is retained in the ER, with dire consequences. The devastating disease results not because the mutation inactivates an important protein but because the active protein is discarded by the cells before it is given an opportunity to function.

Proteins Are Further Modified and Sorted in the Golgi Apparatus

The **Golgi apparatus** is usually located near the cell nucleus, and in animal cells it is often close to the centrosome, a small structure near the cell center. This organelle consists of a collection of flattened, membrane-enclosed sacs (*cisternae*; singular, *cisterna*), which are piled like stacks of plates. Each stack contains 3–20 cisternae (Figure 15–24). The number of Golgi stacks per cell varies greatly depending on the cell type: some cells contain one large stack, while others contain hundreds of very small ones.

Each Golgi stack has two distinct faces: an entry, or *cis*, face and an exit, or *trans*, face. The *cis* face is adjacent to the ER, while the *trans* face points toward the plasma membrane. The outermost cisterna at each face is connected to a network of interconnected membranous tubes and vesicles (see Figure 15–24A). Soluble proteins and membrane enter the *cis Golgi network* via transport vesicles derived from the ER. The proteins travel through the cisternae in sequence by means of transport vesicles that bud from one cisterna and fuse with the next. Proteins exit from the *trans* Golgi network in transport vesicles destined for either the cell surface or another compartment (see Figure 15–17). Both the *cis* and *trans* Golgi networks are thought to be important for protein sorting: proteins entering the *cis* Golgi network can either move onward through the Golgi stack or, if they contain an ER retention signal, be



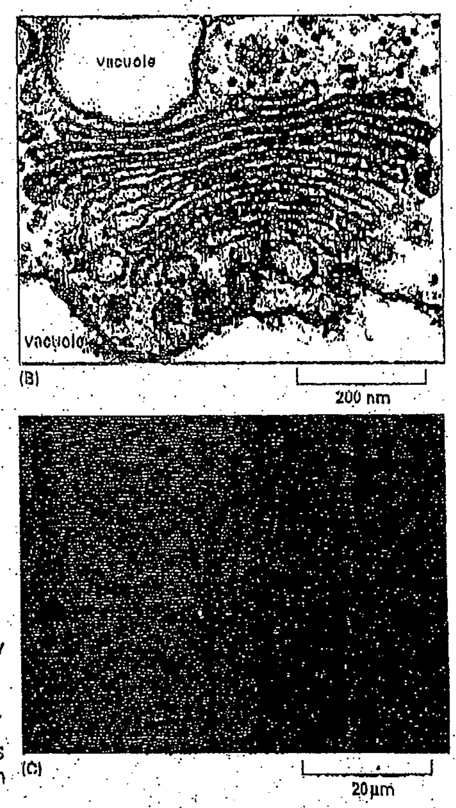


Figure 15–24 The Golgi apparatus is made of a stack of flattened, membrane-enclosed sacs. (A) Three-dimensional reconstruction of a Golgi stack. It was reconstructed from electron micrographs of the Golgi apparatus in a secretory animal cell. (B) Electron micrograph of a Golgi stack from a plant cell, where the Golgi apparatus is especially distinct. The Golgi apparatus is oriented as in (A). (C) The Golgi apparatus in a cultured fibroblast stained with a fluorescent antibody that labels the Golgi apparatus specifically. A *red arrow* indicates the direction of the cell's movement. The *cis* face of the Golgi apparatus is close to the nucleus, and its *trans* face is oriented toward the direction of movement. (A, redrawn from A. Rambourg and Y. Clermont, *Eur. J. Cell Biol.* 51:189–200, 1990; B, courtesy of George Palade; C, courtesy of John Henley and Mark McNiven.)

returned to the ER; proteins exiting the *trans* Golgi network are sorted according to whether they are destined for lysosomes or for the cell surface. We discuss some examples of sorting by the *trans* Golgi network later, and we present some of the methods for tracking proteins through the secretory pathways of the cell in How We Know, pp. 520–521.

Many of the oligosaccharide groups that are added to proteins in the ER undergo further modifications in the Golgi apparatus. On some proteins, for example, complex oligosaccharide chains are created by a highly ordered process in which sugars are added and removed by a series of enzymes that act in a rigidly determined sequence as the protein passes through the Golgi stack. There is a clear correlation between the position of an enzyme in the chain of processing events and its localization in the Golgi stack: enzymes that act early are found in cisternae close to the *cis* face, while enzymes that act late are found in cisternae near the *trans* face.

Secretory Proteins Are Released from the Cell by Exocytosis

In all eucaryotic cells there is a steady stream of vesicles that bud from the *trans* Golgi network and fuse with the plasma membrane. This *constitutive exocytosis pathway* operates continually and supplies newly made lipids and proteins to the plasma membrane; it is the pathway for

How We Know: Tracking protein and vesicle transport

Over the years, biologists have taken advantage of a variety of techniques to untangle the pathways and mechanisms by which proteins are sorted and transported into and out of the cell and its resident organelles. As we saw earlier, transferring an ER signal sequence to a cytosolic protein allowed researchers to confirm that such signal peptides serve to target proteins to specific intracellular compartments—in this example, the ER (see Figure 15–6). But such signal-swapping experiments are not the only way to track a protein's progress through the cell. Biochemical, genetic, and molecular biological and microscopic techniques also provide a means for studying how proteins shuttle from one cellular compartment to another. In some cases, these methods can be used to track the migration of proteins and transport vesicles in real time inside living cells.

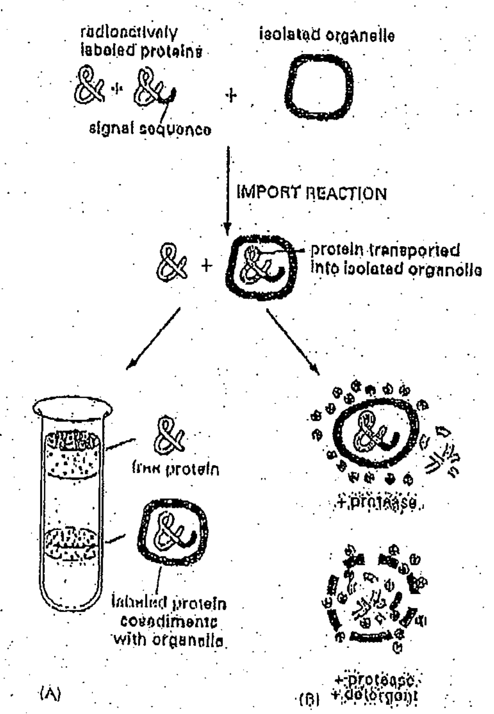


Figure 15–25 Several methods can be used to determine whether a protein bearing a particular signal sequence is transported into a preparation of isolated organelles. (A) The labeled protein with or without a signal sequence is incubated with the organelles and the preparation is centrifuged. Only those labeled proteins that contained a signal sequence will be transported and therefore should co-fractionate with the organelle. (B) The labeled proteins are incubated with the organelle and a protease is added to the preparation. A transported protein will be selectively protected from digestion by the protease; adding a detergent that disrupts the organelle membrane will eliminate that protection and the transported protein will also be degraded.

In a tube

A protein bearing a signal sequence can be introduced to a preparation of isolated organelles in a test tube. This mixture can then be tested to see whether the protein will be taken up by the organelle being examined. The protein is usually produced *in vitro* by cell-free translation of a purified mRNA encoding the polypeptide; in the process, radioactive amino acids can be used to label the protein so that it will be easy to isolate and to follow. The labeled protein is incubated with a selected organelle and its translocation monitored by one of several different methods (Figure 15–25).

Ask a yeast

Movement of proteins between different cellular compartments via transport vesicles has been studied extensively using genetic techniques. Studies of mutant yeast cells that are defective for secretion at high temperatures have identified more than 25 genes that are involved in exocytosis. Many of these mutant genes encode temperature-sensitive proteins that are involved in transport and secretion. These mutant proteins may function normally at 25°C, but when the yeast cells are shifted to 35°C, they are inactivated. As a result, when researchers raise the temperature, proteins destined for secretion instead accumulate inappropriately in the ER, the Golgi apparatus, or transport vesicles (Figure 15–26).

At the movies

Perhaps the most dramatic method for tracking a protein as it moves throughout the cell involves tagging the polypeptide with green fluorescent protein (GFP). Using the genetic engineering techniques discussed in Chapter 10, this small protein can be fused to other cellular proteins. Fortunately, for most proteins studied, the addition of GFP does not perturb the molecule's normal function or transport. The movement of a GFP-tagged protein can then be monitored in a living cell with a fluorescent microscope.

GFP fusion proteins are widely used to study the location and movement of proteins in cells (Figure 15–27). GFP fused to

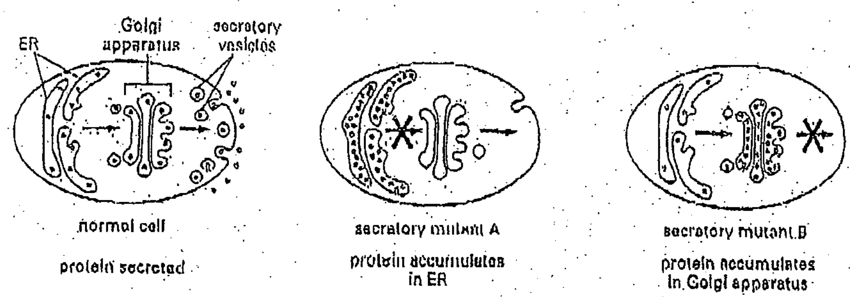


Figure 15–26 Temperature-sensitive mutants have been used to dissect the protein secretory pathway in yeast. Mutations in genes involved at different stages of the transport process result in the accumulation of proteins in the ER, the Golgi apparatus, or other transport vesicles. For example, a mutation A that blocks transport from the ER to the Golgi apparatus will cause a buildup of proteins in the ER. A mutation B that blocks exit of proteins from the Golgi apparatus will cause proteins to accumulate within that organelle.

proteins that shuttle in and out of the nucleus, for example, can be used to study nuclear transport events. GFP fused to plasma membrane proteins can be used to measure the kinetics of their movement through the secretory pathway. Movies demonstrating the power and beauty of this technique are included on the CD that accompanies this book.

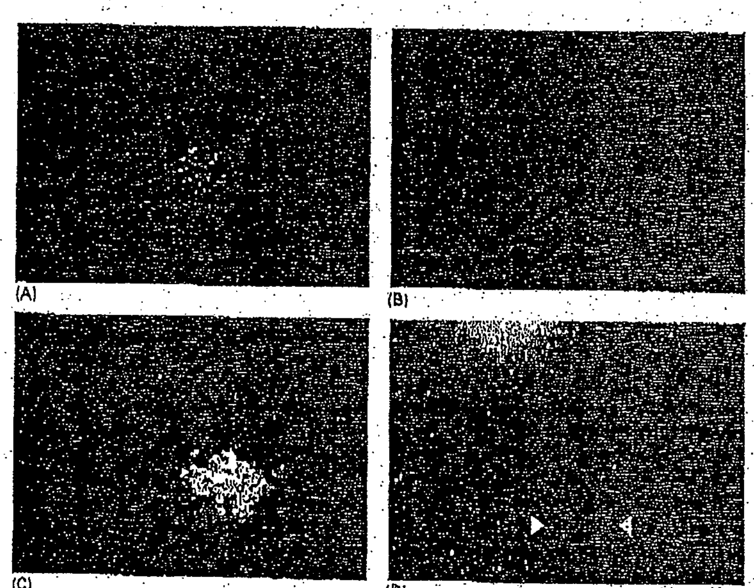


Figure 15–27 GFP fusion allows proteins to be tracked throughout the cell. In this experiment, GFP is fused to a viral coat protein and expressed in cultured cells. In an infected cell, the viral protein will move through the secretory pathway from the ER to the cell surface, where a virus particle would be assembled. The viral coat protein used in this experiment contains a mutation that allows export from the ER only at a low temperature. (A) At high temperatures, the fusion protein labels the ER. (B) As the temperature is lowered, the GFP fusion protein rapidly accumulates at ER exit sites. (C) The fusion protein then moves to the Golgi apparatus. (D) Finally, the fusion protein is delivered to the plasma membrane. The halo between the two arrowheads marks the spot where a single vesicle has fused expelling the viral coat protein into the plasma membrane. (A–D, courtesy of Jennifer Lippincott-Schwartz.)

plasma membrane growth when cells enlarge before dividing. The constitutive pathway also carries proteins to the cell surface to be released to the outside, a process called **secretion**. Some of the released proteins adhere to the cell surface, where they become peripheral proteins of the plasma membrane; some are incorporated into the extracellular matrix; still others diffuse into the extracellular fluid to nourish or to signal other cells. Because entry into this nonselective pathway does not require a particular signal sequence (like the ones that direct proteins to lysosomes or back to the ER), it is sometimes referred to as the *default pathway*.

In addition to the constitutive exocytosis pathway, which operates continually in all eucaryotic cells, there is a *regulated exocytosis pathway*, which operates only in cells that are specialized for secretion. Specialized *secretory cells* produce large quantities of particular products, such as hormones, mucus, or digestive enzymes, which are stored in secretory vesicles for later release. These vesicles bud off from the *trans* Golgi network and accumulate near the plasma membrane. There they wait for the extracellular signal that will stimulate them to fuse with the plasma membrane and release their contents to the cell exterior (Figure 15–28). An increase in blood glucose, for example, signals cells in the pancreas to secrete the hormone insulin (Figure 15–29).

Proteins destined for secretory vesicles are sorted and packaged in the *trans* Golgi network. Proteins that travel by this pathway have special surface properties that cause them to aggregate with one another under the ionic conditions (acidic pH and high Ca^{2+}) that prevail in the *trans* Golgi network. The aggregated proteins are recognized by an unknown mechanism and packaged into secretory vesicles, which pinch off from the network. Proteins secreted by the constitutive pathway do not aggregate and are therefore carried automatically to the plasma membrane by the transport vesicles of the constitutive pathway. Selective aggregation has another function: it allows secretory proteins to be packaged into secretory vesicles at concentrations much higher than the concentration of the unaggregated protein in the Golgi lumen. This increase in concentration can reach up to 200-fold, enabling secretory cells to release large amounts of the protein promptly when triggered to do so (see Figure 15–29).

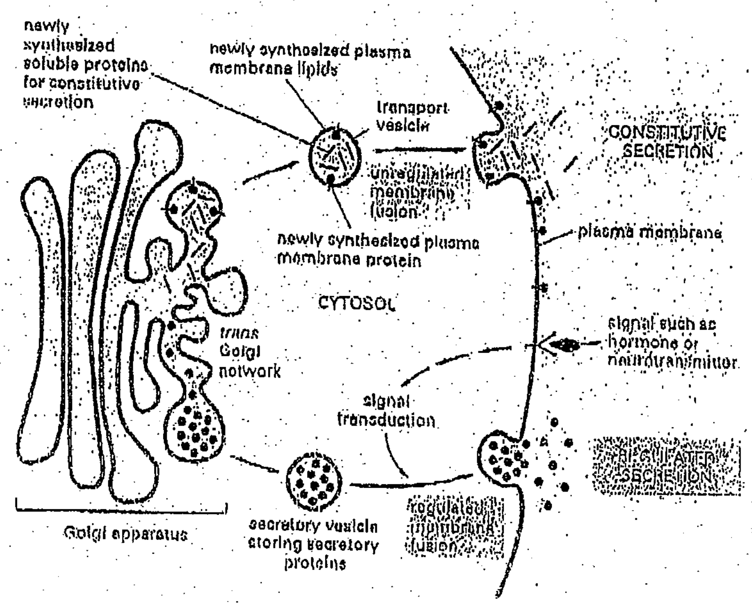


Figure 15–28 In secretory cells, the regulated and constitutive pathways of exocytosis diverge in the *trans* Golgi network. Many soluble proteins are continually secreted from the cell by the constitutive secretory pathway, which operates in all cells. This pathway also continually supplies the plasma membrane with newly synthesized lipids and proteins. Specialized secretory cells have, in addition, a regulated exocytosis pathway, by which selected proteins in the *trans* Golgi network are diverted into secretory vesicles, where the proteins are concentrated and stored until an extracellular signal stimulates their secretion. It is unclear how aggregates of secretory proteins are segregated into secretory vesicles. Secretory vesicles have unique proteins in their membranes; perhaps some of these proteins act as receptors for secretory protein aggregates in the *trans* Golgi network.

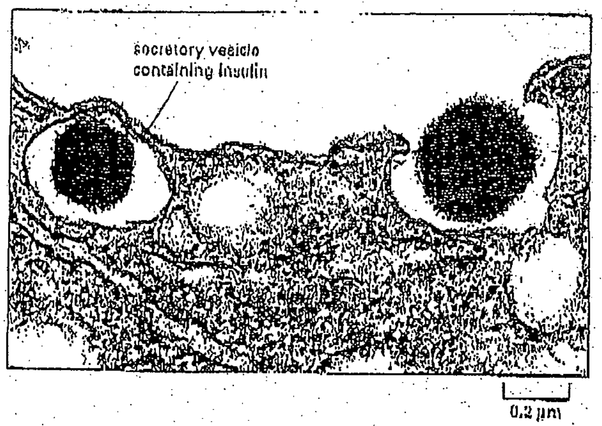


Figure 15–29 Secretory vesicles package and discharge concentrated aggregates of protein. The electron micrograph shows the release of insulin into the extracellular space from a secretory vesicle of a pancreatic β cell. The insulin is stored in a highly concentrated form in each secretory vesicle and is released only when the cell is signaled to secrete by an increase in glucose levels in the blood. (Courtesy of Lelio Orci, from L. Orci, J.D. Vassali, and A. Perrelet, *Sci. Am.* 256:85–94, 1988.)

When a secretory vesicle or transport vesicle fuses with the plasma membrane and discharges its contents by exocytosis, its membrane becomes part of the plasma membrane. Although this should greatly increase the surface area of the plasma membrane, it does so only transiently because membrane components are removed from other regions of the surface by endocytosis almost as fast as they are added by exocytosis. This removal returns both the lipids and the proteins of the vesicle membrane to the Golgi network, where they can be used again.

Question 15–7

What would you expect to happen in cells that secrete large amounts of protein through the regulated secretory pathway if the ionic conditions in the ER lumen could be changed to resemble those in the lumen of the *trans* Golgi network?

Endocytic Pathways

Eucaryotic cells are continually taking up fluid, as well as large and small molecules, by the process of endocytosis. Specialized cells are also able to internalize large particles and even other cells. The material to be ingested is progressively enclosed by a small portion of the plasma membrane, which first buds inward and then pinches off to form an intracellular *endocytic vesicle*. The ingested material is ultimately delivered to lysosomes, where it is digested. The metabolites generated by digestion are transferred directly out of the lysosome into the cytosol, where they can be used by the cell.

Two main types of endocytosis are distinguished on the basis of the size of the endocytic vesicles formed. *Pinocytosis* ("cellular drinking") involves the ingestion of fluid and molecules via small vesicles (<150 nm in diameter). *Phagocytosis* ("cellular eating") involves the ingestion of large particles, such as microorganisms and cell debris, via large vesicles called *phagosomes* (generally >250 nm in diameter). Whereas all eucaryotic cells are continually ingesting fluid and molecules by pinocytosis, large particles are ingested mainly by specialized *phagocytic cells*.

In this final section we trace the endocytic pathway from the plasma membrane to lysosomes. We start by considering the uptake of large particles by phagocytosis.

Specialized Phagocytic Cells Ingest Large Particles

The most dramatic form of endocytosis, phagocytosis, was first observed more than a hundred years ago. In protozoa, phagocytosis is a form of feeding: microorganisms ingest large particles, such as bacteria, by taking them up into phagosomes; these phagosomes then fuse with lysosomes, where the food particles are digested. Few cells in multicellular organisms are able to ingest large particles efficiently. In the animal gut, for example, large particles of food have to be broken down to individual molecules by extracellular enzymes before they can be taken up by the absorptive cells lining the gut.